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Up- and Down-Regulation by Glucocorticoids of the Constitutive Expression of the Mast Cell Growth Factor Stem Cell Factor by Human Lung Fibroblasts in Culture

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ABSTRACT

Stem cell factor (SCF) is a major mast cell growth factor that promotes differentiation and chemotaxis of mast cells and inhibits their apoptosis. SCF therefore may be involved in diseases associated with an increased number of tissue mast cells such as asthma, for which the major treatment is glucocorticoids. In this study, we evaluated the effect of the glucocorticoid budesonide on the constitutive expression of SCF by human lung fibroblasts in primary culture. Budesonide (0.1 μM) induced a time-dependent biphasic effect on SCF mRNA and protein production. A short treatment (2.5–10 hr) induced an inhibition of SCF protein accumulation (−58% at 2.5 hr) and mRNA expression (−69% at 2.5 hr), associated with an accelerated decay of SCF mRNA and with a decrease in SCF gene transcription observed by nuclear run-on assay. Longer treatment (24–72 hr) led to increases in SCF protein accumulation (+64% at 48 hr) and mRNA expression (+125% at 24 hr) as a consequence of transcriptional activation. Similar effects of a decrease followed by an increase in SCF production were observed using another glucocorticoid, dexamethasone. Overall, our results show that glucocorticoids potently regulate SCF expression in human lung fibroblasts, successively decreasing and increasing SCF mRNA levels according to treatment duration. Such time-dependent modulation of SCF levels may explain some current discrepant findings about the effects of glucocorticoids on SCF production and may have functional consequences during glucocorticoid treatment, such as asthma therapy.

SCF, also termed Kit ligand, steel factor, or mast cell growth factor (Huang *et al.*, 1990; Martin *et al.*, 1990; Zsebo *et al.*, 1990), is the ligand of the c-*kit* proto-oncogene product. SCF is expressed in two forms, sSCF and mSCF, after alternative splicing of the sixth exon, which encodes a proteolytic cleavage site (Anderson *et al.*, 1991; Flanagan *et al.*, 1991). In addition to its multipotent role in hematopoiesis and in the development and function of germ cells and melanocytes (Galli, 1990), SCF acts as an important growth factor for human mast cells (Kirshenbaum *et al.*, 1992; Rottem *et al.*, 1994; Galli *et al.*, 1995). It may be involved in pathological increases in number (Iemura *et al.*, 1994; Nilsson *et al.*, 1994; Costa *et al.*, 1996; Kassel *et al.*, 1998) and activation (Bischoff and Dahinden, 1992; Okayama *et al.*, 1995; Costa *et al.*, 1996) of mast cells.

The major treatment for mast cell-associated diseases such as asthma remains glucocorticoids, which reduce mast cell numbers, in particular in the bronchial mucosa of asthmatic patients (Jeffery *et al.*, 1992; Laitinen *et al.*, 1992). Glucocorticoids affect cells in many ways, but their major therapeutic benefit is likely to occur through the regulation of the expression of genes involved in inflammation. For instance, glucocorticoids lower the expression of intercellular adhesion molecule-1, of inducible nitric oxide synthase, and of several proinflammatory cytokines; on the other hand, they also increase the expression of lipocortin 1 and β_2-adrenoceptors (for a review, see Barnes and Adcock, 1993). The hypothesis that glucocorticoids may regulate SCF production by resident cells in the inflamed tissue has been put forward.

A sequence marginally homologous to the GRE has been described in the SCF promoter (Taylor *et al.*, 1996). We hypothesized a possible direct regulation of SCF expression by glucocorticoids. We thus investigated the effect of the glucocorticoid budesonide on SCF constitutive expression by human lung fibroblasts in primary culture and report opposite regulatory effects of glucocorticoids on SCF protein and mRNA expression in function of time.

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ABBREVIATIONS: SCF, stem cell factor; AP-1, activating protein-1; FCS, fetal calf serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GR, activated complex glucocorticoid-glucocorticoid receptor; ELISA, enzyme-linked immunosorbent assay; RT, reverse transcription; PCR, polymerase chain reaction; GRE, glucocorticoid response element; sSCF, soluble stem cell factor; mSCF, membrane-bound stem cell factor.

Materials and Methods

Culture of Human Lung Fibroblasts

Human lung-derived fibroblasts were obtained by the explants technique. Briefly, macroscopically normal human lung tissue obtained within 1 hr of resection for bronchocarcinoma was separated into fragments of 0.5–2 mm^3. Explants were washed three times for 10 min each at 37° with Eagle's minimum essential medium (GIBCO BRL, Cergy Pontoise, France), which contained 10% FCS, 150 units/ml penicillin, 150 μg/ml streptomycin, 100 μg/ml gentamicin, and 1 μg/ml fungizone (GIBCO BRL). They then were plated onto 35-mm tissue culture dishes (Costar, Acton, MA), dried for 10 min at room temperature to facilitate adhesion, and subsequently cultured in the same medium in a humidified mixture of 95% air and 5% CO_2 at 37° with the medium changed twice a week. The explants were removed after 2–3 weeks, and the cells were allowed to reach confluence within 1–2 additional weeks. The cells then were trypsinized for 5 min, resuspended in Dulbecco's modified Eagle's medium/F-12 (1:1) (GIBCO BRL); supplemented with 10% FCS, 50 units/ml penicillin, and 50 μg/ml streptomycin; and replated in 25-cm^2 tissue culture flasks (Costar). They were characterized as fibroblasts morphologically and by immunocytochemistry using an anti-fibroblast monoclonal antibody (5B5) that reacted with the β subunit of prolyl-4-hydroxylase (DAKO, Trappes, France). They subsequently were split 1:4 at confluence and passaged. Fibroblasts were used at passages 5–7.

Glucocorticoid Treatment

At confluence, fibroblasts were placed into a quiescent state by reducing the FCS content to 0.3% for 24 hr. Budesonide (kindly provided by Dr. R. Brattsand, Astra, Lund, Sweden), prepared from 10 mM stock solution in absolute ethanol, was added at the indicated concentrations. Dexamethasone, another glucocorticoid (Sigma Chemical, St Louis, MO), was used in similar conditions. Cells were incubated for the indicated period of time at 37° in humidified 95% air/5% CO_2. Control cells were incubated in the same conditions with solvent alone. Experiments also were performed using a different protocol where treatment start was staggered to conclude at the same time. Supernatants were sampled and stored at −70° until assayed for SCF. Fibroblasts were harvested for total RNA extraction.

SCF ELISA

Immunoreactive SCF released in the supernatant of solvent- and glucocorticoid-treated fibroblasts was quantified by a sensitive ELISA procedure, with a capture anti-human SCF monoclonal antibody (clone 13306.6; R&D Systems Europe, Abingdon, UK) and a biotinylated detection anti-human SCF polyclonal antibody (R&D Systems Europe), revealed by extravidin-horseradish peroxidase (Sigma) and a 3,3′,5,5′-tetramethylbenzidine liquid substrate system (Sigma Chemical). Standard curves were generated with recombinant human SCF (R&D Systems Europe) diluted in culture medium containing 0.3% FCS (i.e., from 3.906 to 250 pg/ml).

Extraction of Total RNA and RT

Total RNA was extracted from solvent- and glucocorticoid-treated fibroblasts with TriReagent (Molecular Research Center, Cincinnati, OH). Isolated RNA was diluted in RNase-free water and quantified by absorbance measurement at 260 nm.

Next, 4 μg of total RNA was incubated with 0.5 μg of random primers for 5 min at 70° and then allowed to cool down at room temperature. RNA subsequently was reverse transcribed in 1× RT buffer [75 mM KCl, 15 mM $MgCl_2$, 10 mM dithiothreitol, and 50 mM Tris, pH 8.5, containing 1 unit/μl RNasin ribonuclease inhibitor, 1 mM concentration of each dNTP, and 10 unit/μl RNase H (−)-Moloney leukemia virus reverse transcriptase (all reagents from Promega, Madison, WI)]. The reaction was conducted for 1 hr at 37°, and the reverse transcriptase was inactivated by heating at 98° for 5 min.

Quantification of SCF cDNA by Competitive PCR

PCR conditions. SCF cDNA was amplified by PCR using primers located upstream of the alternatively spliced 6th exon, thus leading to a single 149-bp PCR product for total SCF. PCR reactions were performed in 1× PCR buffer (50 mM KCl; 10 mM Tris·HCl, pH 8.3) containing 1.5 mM $MgCl_2$, 0.2 mM dNTP, 10 pmol of each primer (sense primer: 5′-TGGATAAGCGAGATGGTAGT-3′; antisense: 5′-TTTTCTTTCACGCACTCCAC-3′), and 2 units of *Taq* DNA polymerase (Promega) in a 50-μl final volume for 40 cycles, with each consisting of 60-sec denaturation at 94°, 30-sec annealing at 50°, and 60-sec extension at 72°. GAPDH cDNA was amplified by PCR in 1× PCR buffer containing 2.5 mM $MgCl_2$, 0.2 mM dNTP, 20 pmol of each primer (sense: 5′-GGTGAAGGTCGGAGTCAACGGA-3′; antisense: 5′-GAGGGATCTCGCTCCTGGAAGA-3′), and 1 unit of *Taq* polymerase in a 50-μl final volume for 30 cycles, each consisting of 60-sec denaturation at 96°, 60-sec annealing at 60°, and 60-sec extension at 72°.

Construction of competitor cDNAs. SCF competitor cDNA was constructed by PCR, introducing a 25-bp deletion in the SCF cDNA sequence. Briefly, SCF 149-bp PCR product was purified after 2% agarose gel electrophoresis, with the QIAEX kit (Qiagen, Courtaboeuf, France). It was reamplified with the 20-bp antisense primer used above and a 40-bp composite sense primer (5′-sense primer, CTTCGTGACAAGTTTTCAAA-3′), made up of the 20-bp sense primer used above, attached to a 20-bp sequence complementary to the sequence located 25 bp downstream in the SCF cDNA. Amplification with these primers led to a 25-bp deletion in the amplified cDNA (SCF-Δ25). PCR was conducted in 1× PCR buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTP, 40 pmol of each primer, and 2 units of *Taq* polymerase in a 50-μl final volume, for 20 cycles, each consisting of 45-sec denaturation at 96°, 60-sec annealing at 50°, and 60-sec extension at 72°. The resulting SCF-Δ25 (124 bp) was resolved on a 2% agarose gel and purified with QIAEX. This cDNA was then reamplified as described for SCF cDNA amplification. Then, it was purified with QIAEX, quantified by absorbance measurement to constitute the stock of SCF-Δ25 competitor, divided into aliquots, and stored at −20°.

The same strategy was used to construct the GAPDH competitor cDNA, using the 20-bp antisense GAPDH-specific primer used above and a 40-bp composite sense primer (5′-sense primer, CCAGGGCTTTTAACTCTGG-3′); the additional 19-bp sequence was complementary to the sequence located 25 bp downstream in the GAPDH cDNA. Amplification with these primers led to a 25-bp deletion in the amplified cDNA (GAPDH-Δ25). PCR amplification was conducted in 1× PCR buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTP, 20 pmol of each primer, and 1 unit of *Taq* polymerase in a 50-μl final volume for 20 cycles, each consisting of 60-sec denaturation at 96°, 60-sec annealing at 60°, and 60-sec extension at 72°. The resulting 215-bp GAPDH-Δ25 PCR product was resolved on a 2% agarose gel and purified with QIAEX. This cDNA then was reamplified as described for GAPDH cDNA amplification. Next, it was purified with QIAEX, quantified by absorbance measurement to constitute the stock of GAPDH-Δ25 competitor, divided into aliquots, and stored at −20°.

Competitive PCR. For SCF competitive PCR, 1 μl of a 1:20 dilution of the RT product was added to individual tubes containing increasing amounts of SCF-Δ25 (0.02, 0.04, 0.08, 0.2, 0.4, and 0.8 amol), and PCR amplification was performed as described. For GAPDH competitive PCR, 1 μl of a 1:200 dilution of the RT product was added to individual tubes containing increasing amounts of GAPDH-Δ25 (0.04, 0.08, 0.2, 0.4, 0.8, and 2 amol), and PCR amplification was performed as described previously.

PCR products analysis. PCR products (15 μl) were denatured by heating at 99° for 10 min in the presence of 50% deionized formamide and resolved by electrophoresis on 50% urea/17% form-

amide/10% polyacrylamide gels in parallel with a 25-bp DNA Ladder (GIBCO BRL). Gels were stained with ethydium bromide, digitalized under UV light with a high performance charge-coupled device camera (Cohu, San Diego, CA), and analyzed with the public domain NIH Image program (written by W. Rasband at the National Institutes of Health and available by anonymous FTP from *ftp://zippy.nimh.nih.gov*). Ratios of SCF to SCF-Δ25 and of GAPDH to GAPDH-Δ25 PCR products were calculated for each SCF-Δ25 and GAPDH-Δ25 concentration. Concentration-ratio curves were constructed to calculate the initial SCF and GAPDH cDNAs concentrations at a ratio of 1.

Expression of results. To normalize for differences in RNA extraction and RT efficiencies, ratios of SCF cDNA/GAPDH cDNA concentrations were calculated in each sample. GAPDH mRNA expression remained unchanged over time and throughout glucocorticoid treatment. Results are expressed as amol SCF cDNA/fmol GAPDH cDNA.

Relative Expression of sSCF and mSCF mRNA

The expression of mRNAs of the two forms of SCF in solvent- and budesonide-treated fibroblasts was studied by PCR amplification after RT. We used primers spanning the alternatively spliced sixth exon (sense primer: 5′-TGGATAAGCGAGATGGTAGT-3′; antisense: 5′-AGCCACAATTTACACTTCTT-3′) to generate a 627-bp product from base 388 to base 1015 for sSCF cDNA and a 544-bp PCR product from base 388 to base 932 for mSCF cDNA, from the sequence reported by Martin *et al*. (1990). Equal amounts of total SCF cDNA, as determined by competitive PCR, were amplified by PCR in 1× PCR buffer containing 2.5 mM $MgCl_2$, 0.1 mM dNTP, 10 pmol of each primer, and 1 unit of *Taq* DNA polymerase in a 50-μl final volume for 30 cycles, each consisting of 60-sec denaturation at 94°, 30-sec annealing at 55°, and 60-sec extension at 72°. After PCR, 15 μl of each reaction product was denatured by heating at 99° for 10 min in the presence of 50% deionized formamide and resolved by electrophoresis on 50% urea/17% formamide/6% polyacrylamide gels in parallel with a 100-bp DNA Ladder (GIBCO BRL). Gels were stained with ethydium bromide and analyzed as described above. The ratio of sSCF to mSCF cDNA then was calculated. The ratio remained constant during the exponential phase of PCR amplification.

Transcript Stability Analysis

Confluent quiescent fibroblasts were treated with budesonide (0.1 μM) or solvent for 2.5 or 24 hr. Supernatants were replaced by 0.3% FCS culture medium containing 5 μg/ml actinomycin D (Sigma Chemical) for periods of time ranging from 0 to 6 hr, as described previously (Haddad *et al*., 1995). Total RNA was isolated in each sample, and SCF mRNA was quantified after RT by competitive PCR. The decay of SCF mRNA was assessed by the calculated half-life values.

Nuclear Run-On Assay

***In vitro* transcription.** Nuclei were prepared from solvent- and budesonide- (0.1 μM) treated (2.5 or 24 hr) fibroblasts by lysing cell membrane in 10 mM Tris·HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% IGEPAL CA-630 (Sigma Chemical), 2.75 mM dithiothreitol, and 20 units/ml RNasin on ice. After centrifugation, isolated nuclei were resuspended in the same buffer without IGEPAL CA-630 and counted. Each reaction (final volume, 400 μl) was carried out in the presence of 5×10^7 isolated nuclei, 40 mM Tris·HCl, pH 8.3, 150 mM NH_4Cl, 7.5 mM $MgCl_2$, 0.625 mM ATP, 0.313 mM GTP, 0.313 mM CTP, 0.3 mCi of [α-^{32}P]UTP (800 Ci/mmol) (DuPont-New England Nuclear, Boston, MA), and 120 units/ml RNasin. Transcription reactions were allowed to proceed for 30 min at 27° before the addition of 40 units of RNasin and 75 units of RQ-1 DNase (Stratagene, La Jolla, CA). After DNase treatment, the radiolabeled RNA that was formed was purified by phenol-chloroform extraction and precipitated three times with ethanol in the presence of 1.33 M ammonium acetate.

SCF and GAPDH cDNA probe synthesis. SCF and GAPDH PCR products (149 and 240 bp, respectively), obtained as described above, were resolved on 2% agarose gel, extracted with QIAEX, and subcloned in pGEM-T plasmid (Promega). Sequence analysis (Génome Express, Paris, France) revealed a 100% homology with the human SCF and GAPDH genes and no significant homology with any other known gene. Sequence comparison with gene databanks used the NCBI Blast program.

Hybridization and quantification. Each sample of radiolabeled RNA was hybridized on individual membranes (Duralon.UV, Stratagene), on which 10 μg of either pGEM-T plasmid (as control) or plasmid-containing inserts of human SCF cDNA or GAPDH cDNA had been immobilized. After hybridization for 72 hr at 42°, the membranes were washed at a final stringency of 0.1× standard saline citrate and 0.1% sodium dodecyl sulfate at 55°, including a 30-min digestion with 1 μg/ml RNase A and 10 units/ml RNase T_1 (Boehringer-Mannheim, Mannheim, Germany) at 37° to digest any single-stranded RNA not hybridized to DNA. After radioautography, films were digitalized, and spots were quantified with NIH Image. The rate of SCF gene transcription was expressed as the ratio of SCF mRNA to GAPDH mRNA signals.

Statistical Analysis

Results are expressed as mean ± standard error. Wilcoxon signed rank test was used for statistical analysis of the results. Values of $p < 0.05$ were considered significant.

Results

Time-dependent biphasic effect of budesonide on SCF production. Kinetic studies were used to analyze the effect of budesonide (0.1 μM) on SCF mRNA and protein expression in human lung fibroblasts in culture (Fig. 1). Budesonide first rapidly and transiently inhibited SCF mRNA expression. Maximum inhibition occurred at 2.5 hr after treatment began (−69%; 19.3 ± 5.5 and 5.9 ± 1.7 amol/fmol GAPDH for control and budesonide-treated fibroblasts, respectively; $p < 0.05$, three experiments). This inhibitory effect progressively decreased over time, and SCF

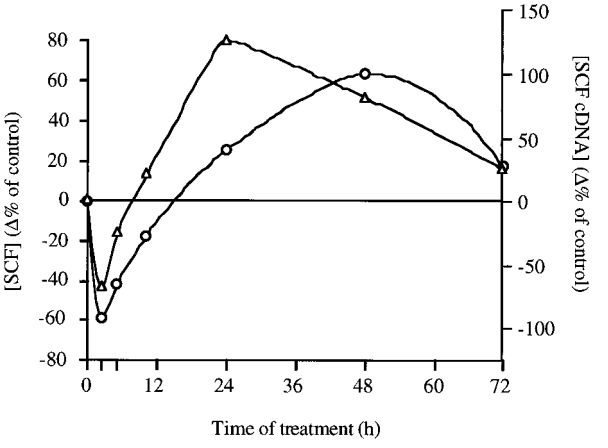

Fig. 1. Kinetic study of the effect of budesonide on SCF protein and mRNA expression by human lung fibroblasts in culture. Fibroblasts were treated with 0.1 μM budesonide for the indicated time. SCF protein levels were assessed in the supernatant by ELISA. SCF cDNA was quantified after total RNA extraction and RT by competitive PCR as described in the text. Results were normalized to GAPDH, which did not vary over time. Results are expressed as percent of variation (Δ%) of SCF protein levels (○, *left axis*) or SCF cDNA levels (△, *right axis*) from fibroblasts treated with solvent alone for the same period of time. Results are mean values of three different experiments performed on fibroblasts from three different donors.

mRNA expression returned to basal level at 5–10 hr. This was followed by stimulated SCF mRNA expression, which was greatest at 24 hr (+125%; 33.4 ± 6.0 and 70.4 ± 13.5 amol/fmol GAPDH for control and budesonide-treated fibroblasts, respectively; $p < 0.05$, three experiments), after which it slowly returned toward basal levels, although still upregulated at 72 hr.

SCF protein production, like SCF mRNA expression, was modulated by budesonide (0.1 μM), also biphasically (Fig. 1). Inhibition of SCF protein production was assessed by measuring the decreased SCF accumulation in the culture supernatant and comparing it with that of the control. Inhibition was greatest at 2.5 hr (−58%; 7.6 ± 0.7 and 3.5 ± 0.6 pg/ml SCF for control and budesonide-treated fibroblasts, respectively; $p < 0.05$, three experiments) and decreased to return to the basal level of expression at 10–24 hr; SCF protein production then increased. The stimulatory effect of budesonide was greatest at 48 hr (+64%; 43.5 ± 2.8 and 71.1 ± 4.9 pg/ml for control and budesonide-treated fibroblasts, respectively; $p < 0.05$, three experiments), after which SCF production slowly returned toward base-line at 72 hr (80.4 ± 8.0 and 95.0 ± 12.2 pg/ml for control and budesonide-treated fibroblasts, respectively; p = NS).

Similar results were obtained when budesonide treatments were staggered to stop the cell culture at the same time, ensuring that the observed biphasic effect of budesonide was not due to a modification in the cell phenotype over time.

Concentration-dependent effect of glucocorticoids on SCF protein production by fibroblasts. A dose-response study was performed at the time of budesonide-induced maximal effects (i.e., at 5 hr for inhibition and at 48 hr for increased expression of SCF protein). A 5-hr treatment with budesonide resulted in a dose-dependent inhibition of SCF protein production (Fig. 2A). Maximum inhibition occurred at 0.1 μM (−48%; 11.4 ± 0.7 and 5.9 ± 0.7 pg/ml SCF for control and budesonide-treated fibroblasts, respectively; $p < 0.05$, three experiments), with an EC_{50} value of 2.5 ± 0.2 nM.

At 48 hr, SCF levels in the culture medium were 3.7 times greater than that at 5 hr (Fig. 2B). Treatment with budesonide resulted in a dose-dependent increase in SCF production (Fig. 2B). The maximum stimulatory effect occurred at 0.1 μM (+81%; 42.6 ± 3.6 and 77.2 ± 2.9 pg/ml SCF observed for control and budesonide-treated fibroblasts, respectively; $p < 0.05$, three experiments), with an EC_{50} value of 2.3 ± 0.3 nM. The time-dependent biphasic effect of budesonide on SCF expression was confirmed with dexamethasone (not shown).

Effect of budesonide on the relative expression of sSCF and mSCF mRNA. Effect of budesonide was analyzed on the relative expression of the two forms of SCF mRNA by RT-PCR, using primers spanning the alternatively spliced 6th exon. No modification of this relative expression was observed, neither at 2.5 hr for maximal inhibition nor at 24 hr for maximal increased expression of SCF mRNA (Fig. 3).

Effect of budesonide on SCF mRNA stability. SCF mRNA stability was studied by measuring SCF mRNA half-life in solvent- and budesonide-treated fibroblasts to which actinomycin D was added (Fig. 4). Before this actinomycin D addition, levels of SCF mRNA expression were 7.5 and 3.2 amol/fmol GAPDH at 2.5 hr and 21.3 and 40.9 amol/fmol GAPDH at 24 hr for control and budesonide-treated fibroblasts, respectively. At 2.5 hr, the estimated half-life of SCF mRNA was 6.2 hr in control fibroblasts treated with solvent alone and 3.5 hr in fibroblasts treated with budesonide (0.1 μM) (Fig. 4A), indicating a glucocorticoid-induced reduction in mRNA stability after treatment.

At 24 hr, half-life decreased to 3.1 hr in control cells, which suggests that SCF mRNA stability decreases as a function of

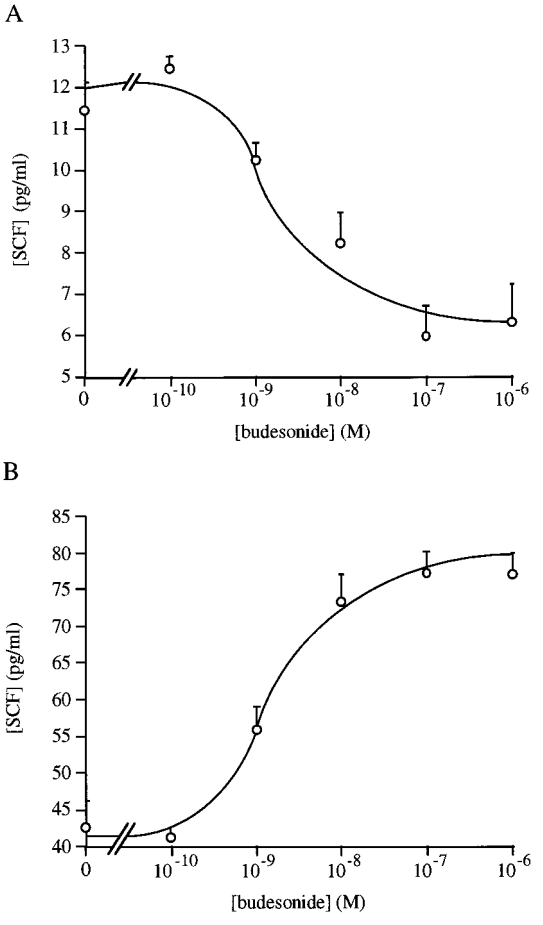

Fig. 2. Concentration-dependent effect of budesonide on SCF protein production by human lung fibroblasts in culture after a 5-hr (A) or 48-hr (B) treatment. SCF levels in the culture supernatants were assessed by ELISA and are expressed in picograms per milliliter. Results are mean ± standard error of three different experiments performed on fibroblasts from three different donors.

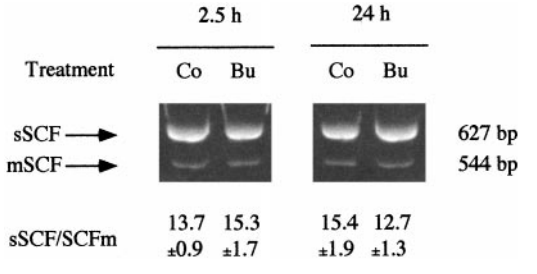

Fig. 3. Effect of budesonide on the relative expression of sSCF and mSCF mRNA in human lung fibroblasts in culture. Total RNA was extracted and reverse transcribed from fibroblasts treated with solvent (*Co*) or fibroblasts treated with 0.1 μM budesonide (*Bu*) for 2.5 or 24 hr. sSCF and mSCF cDNAs were amplified by PCR in RT products containing equal amounts of total SCF cDNA quantified by competitive PCR. Ratios of sSCF to mSCF are expressed as mean ± standard error of three different experiments performed on fibroblasts from three different donors.

time. Treatment with budesonide (0.1 μM) had no effect, at that time, on SCF mRNA half-life (3.2 hr) (Fig. 4B).

Effect of budesonide on the rate of SCF gene transcription. Nuclear run-on assays were performed on fibroblasts treated with budesonide (0.1 μM) or solvent for 2.5 and 24 hr. At 2.5 hr, the rate of SCF gene transcription in fibroblasts treated with budesonide, expressed as the ratio of SCF gene to GAPDH gene transcription, was 48% of the rate in fibroblasts treated with solvent alone (Fig. 5A).

At 24 hr, in control cells, SCF gene transcription rate was lower than that at 2.5 hr, suggesting a decrease in SCF gene transcription over time. Treatment with budesonide (0.1 μM) tripled the SCF gene transcription rate compared with that in control fibroblasts (Fig. 5B).

Discussion

We present here evidence that glucocorticoids exert a concentration- and time-dependent effect on the constitutive production of SCF protein and mRNA by human lung fibroblasts in culture, with no modification of the relative expression of both forms of SCF transcripts. A short treatment inhibited basal SCF production by decreasing both SCF mRNA stability and SCF gene transcription. Conversely, a longer treatment enhanced SCF expression by increasing SCF gene transcription.

The late up-regulation of SCF production after 24–72 hr of glucocorticoid treatment agrees with results observed in other cell types, such as human bone marrow stromal fibroblasts (Linenberger *et al*., 1995) or permanent human bone marrow stromal cell lines (Thalmeier *et al*., 1996). In both of these cell types, a 12–24-hr treatment with glucocorticoids induced a 3–4-fold increase in constitutive SCF mRNA expression (Linenberger *et al*., 1995; Thalmeier *et al*., 1996). However, some other findings are opposed to these and ours. In particular, Haynesworth *et al*. (1996) found no effect of glucocorticoids at 24 hr on human marrow-derived mesenchymal progenitor cells. Furthermore, Finotto *et al*. (1997) reported decreased SCF expression at 72 hr in human fetal skin fibroblasts, daily restimulated with glucocorticoids in FCS-rich culture medium, as opposed to our single glucocorticoid treatment on quiescent fibroblasts. Thus, whether glucocorticoids enhance or decrease SCF expression might strongly depend on the cellular context.

The mechanism by which the late increased SCF production occurs includes increased expression of SCF mRNA, which was unrelated to SCF mRNA stabilization. This was rather related to a transcriptional activation because budesonide markedly increased SCF gene transcription rate. In the SCF promoter, a sequence marginally homologous to the GRE has been described (Taylor *et al*., 1996). Thus, the binding of the activated complex GR to this DNA sequence would either directly stimulate transcription or act in synergy with transcription factors involved in the basal level of SCF gene promoter activity, such as AP-1, AP-2, or SP1 (Taylor *et al*., 1996). This mechanism has been described for

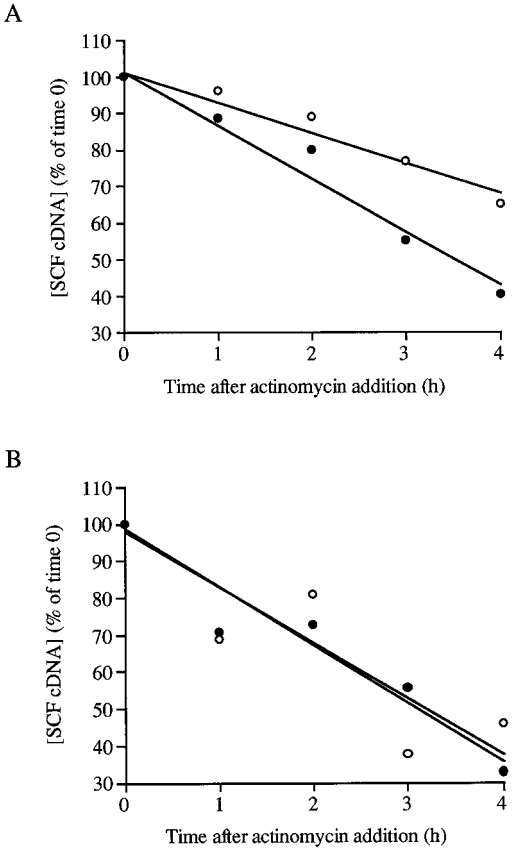

Fig. 4. Effect of budesonide on SCF mRNA stability. Fibroblasts were treated with solvent (○, control) or with 0.1 μM budesonide (●) for (A) 2.5 hr or (B) 24 hr. Fibroblasts then were washed and treated with 5 μg/ml actinomycin D for the indicated time, and SCF cDNA was quantified by competitive PCR. Results are expressed as percent of control SCF cDNA obtained before actinomycin D addition (time 0). Data are from one representative experiment of three performed on fibroblasts from three different donors.

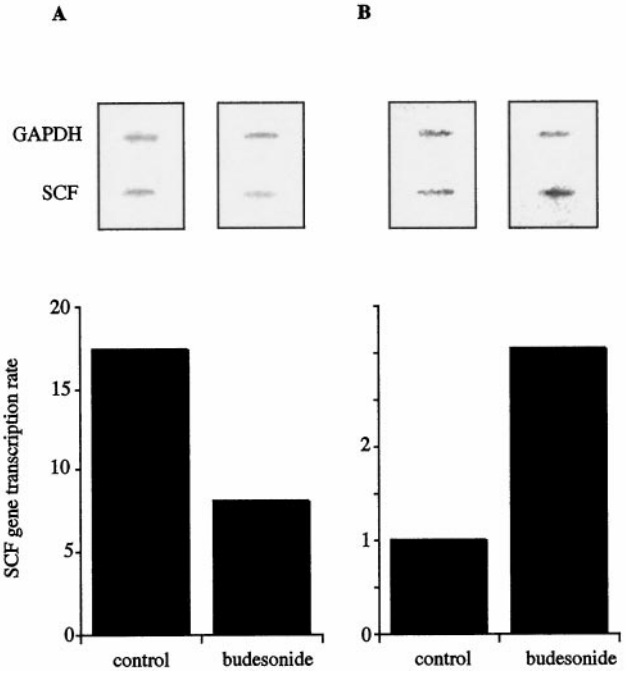

Fig. 5. Effect of budesonide on SCF mRNA transcription rate. Fibroblasts were treated with solvent (*control*) or with 0.1 μM budesonide for (A) 2.5 hr or (B) 24 hr. Nuclei were prepared, and the rate of SCF gene transcription was assessed. *Top*, autoradiography of blots. *Bottom*, SCF gene transcription rate is expressed as the ratio of SCF mRNA signal to GAPDH mRNA signal.

many other genes (Strahle *et al.*, 1988; Barnes and Adcock, 1993). However, an indirect activation of SCF gene transcription through increased/decreased expression by glucocorticoids of some activator/repressor of SCF gene transcription cannot be excluded. In addition, the observed increase in SCF protein expression remained moderate (60%) compared with the marked increase of SCF gene transcription rate (3-fold). This may indicate that some intermediary steps counterbalancing strong variations in SCF levels are involved in the glucocorticoid-induced regulation of SCF expression.

In contrast, an early down-regulation of SCF production by glucocorticoids was observed. This finding is totally consistent with a study of nasal polyp-derived fibroblasts, where budesonide reduces basal SCF production after a 4-hr treatment (Kim *et al.*, 1997). The reduced SCF production by lung fibroblasts was associated with a decrease in the SCF mRNA level. This reduction, in turn, was associated with SCF mRNA destabilization, as was seen from the decreased half-life of mRNA in cells treated with the transcription blocker actinomycin D. The glucocorticoids here seem to accelerate a process that normally occurs later: in the untreated control cells, the half-life of SCF mRNA after 24 hr was similar to that of cells treated for 2.5 hr with budesonide. The human SCF mRNA sequence (Martin *et al.*, 1990) does not exhibit AU-rich elements in its 3′-untranslated region, which are involved in glucocorticoid-induced decrease in mRNA stability (Peppel *et al.*, 1991; Ritsimaki *et al.*, 1996). The observed decrease in SCF mRNA half-life after a 2.5-hr treatment with budesonide therefore must be related to other mechanisms, possibly the regulation of poly(A)$^+$ binding proteins that protect mRNA from degradation (Ross, 1995).

The early decrease in SCF mRNA level also was related to a repression of SCF gene transcription, shown by the decreased rate of transcription in cells treated for 2.5 hr by glucocorticoids. The promoter sequence of the SCF gene (Taylor *et al.*, 1996) does not exhibit consensus negative GRE sequences, which may be involved in the negative regulation of transcription by glucocorticoids (Sakai *et al.*, 1988; Cairns *et al.*, 1993). The observed inhibition of basal SCF gene transcription therefore might imply some other mechanisms, such as an interaction of activated GR with the transcription factors involved in the basal activity of SCF gene promoter (Taylor *et al.*, 1996). This mechanism has been described, in particular, for AP-1 (for reviews, see Ponta *et al.*, 1992; Barnes and Adcock, 1993; Pfahl, 1993), and SP1, whose DNA binding activity is blocked by dexamethasone (Jarvis and Qureshi, 1997). Such a transcriptional repression by glucocorticoids in now well documented for several other genes, including proinflammatory cytokines and chemokines (Ponta *et al.*, 1992; Barnes and Adcock, 1993; Pfahl, 1993).

Several lines of evidence suggest that the transactivation and repression functions of the glucocorticoid receptor may be independent (Ponta *et al.*, 1992). For instance, point mutations in the DNA binding domain of the glucocorticoid receptor inhibit the glucocorticoid-induced transrepression of a reporter gene activated by AP-1, without affecting the glucocorticoid-induced transactivation of a GRE-containing reporter gene (Heck *et al.*, 1994). In addition, new synthetic glucocorticoids have been developed that strongly repress an AP-1-activated reporter gene, with little or no transactivation of a GRE-containing reporter gene (Vayssière *et al.*, 1997). This strongly suggests that transactivation and transrepression are two separable functions of the glucocorticoid receptor. Therefore, the successive inhibition and stimulation of SCF gene transcription induced by budesonide might arise from two independent mechanisms, occurring either successively or concomitantly with the successive predominance of one or the other as a function of time of glucocorticoid treatment. A relevant model is that proposed by Diamond *et al.* (1990) to explain how the interaction of GR with the transcription factor AP-1 evokes both repression and activation of proliferin gene transcription. The interaction of GR with Jun-Fos heterodimers would lead to transrepression, and interaction with c-Jun homodimers would lead to transactivation (Diamond *et al.*, 1990). Thus, whether the glucocorticoid regulation of transcription of a single gene is negative or positive would depend on the cellular context (Diamond *et al.*, 1990; Zhang *et al.*, 1991; Pearce *et al.*, 1993). In our study, the responsiveness of fibroblasts was modified by a long glucocorticoid treatment in such a way that the treatment then induces the activation of SCF gene transcription. Our observations, together with the apparently discrepant findings about the regulation of SCF expression discussed above, lead to propose that regulation of SCF expression by glucocorticoids may strongly depend on the physiological state of the target cell.

In conclusion, our study shows that glucocorticoids have time-dependent opposite regulatory effects on the constitutive SCF production by human lung fibroblasts. A short treatment destabilized SCF mRNA and inhibited SCF gene transcription, and a longer treatment stimulated SCF gene transcription. These transcriptional and post-transcriptional regulations of SCF expression may depend highly on the cellular context. This might have important consequences for the management of diseases such as asthma, which are associated with a local increased number and activation of tissue mast cells.

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